

**U.S.S.N. 09/030,571**

**Cantor *et al.***

**AMENDMENT**

**AMENDMENTS TO THE SPECIFICATION**

**Please replace the paragraph on page 46, line 19 through page 47, line 9 with the following amended paragraph:**

**Oligonucleotide ligation after target hybridization.** Stacking hybridization without ligation has been demonstrated in a simple format. Eight-mer oligonucleotides were annealed to a target and then annealed to an adjacent 5-mer to extend the readable sequence from 8 to 13 bases. This is done with small pools of 5-mers specifically chosen to resolve ambiguities in sequence data that has already been determined by ordinary sequencing by hybridization using 8-mers alone. The method appears to work quite well, but it is cumbersome because a custom pool of 5-mers must be created to deal with each particular situation. In contrast, the approach taken herein (FIG. 10 FIG. 9), after ligation of the target to the probe, is to ligate a mixture of 5-mers arranged in polychromatically labeled orthogonal pools. For example, using 5-mers of the form pATGCAp or pATGCddA, only a single ligation event will occur with each probe-target complex. These would be 3' labeled to avoid interference with the ligase. Only ten pools are required for a binary sieve analysis of 5-mers. In reality it would make sense to use many more, say 16, to introduce redundancy. If only four colors are available, those would require four successive hybridizations. For example, sixteen colors would allow a single hybridization. But the result of this scheme is that one reads ten bases per site in the array, equivalent to the use of  $4^{10}$  probes, but one only has to make  $2 \times 4^5$  probes. The gain in efficiency in this scheme is a factor of 500 over conventional sequencing by hybridization.

**Please replace the paragraph on page 47, lines 11-27 with the following amended paragraph:**

**Synthesis of custom arrays of probes.** Custom arrays of probe would be useful to detect a change in nucleic acid sequence, such as any single base change in a pre-selected large population of sequences. This is important for detecting mutations, for comparative sequencing, and for finding new, potentially rare polymorphisms. One set of target sequences can be customized

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to an initial general array of nucleic acid probes to turn the probe into a specific detector for any alterations of a particular sequence or series of sequences. The initial experiment is the same as outlined above in Example 5 Example 4, except that the 3'-blocked 5-mers are unlabeled. After the ligation, the initial nucleic acid target strand along with its attached 18 nucleotide stalk is removed, and a new unligated 18 nucleotide stalk annealed to each element of the immobilized array (FIG. 11). The difference is that because of its history, many (ideally 50% or more), of the elements of that array now have 10 base 3' extensions instead of 5 base extensions. These do not represent all  $4^{10}$  possible 10-mers, but instead represent just those 10-mers which were present in the original sample. A comparison sample can now be hybridized to the new array under conditions that detect single mismatches in a decanucleotide duplex. Any samples which fail to hybridize are suspects for altered bases.